Significance of Various Type Chromosome Aberrations for Man

by Warren W. Nichols*

A goal of mutagenicity testing is to develop a test that detects genetic damage and mutations with great sensitivity, and to have this test relevant to man. No test available at the present time completely fulfills this goal, and a variety of compromises have been and continue to be made in test systems.

In considering cytogenetic techniques it is quite clear that these are excellent methods for detecting chromosomal abnormalities. Changes in chromosome number and translocations that can be heritable can be detected and are significant to the human population. This is clear enough that it does not require further discussion. This does not mean that all details of this methodology are established, but there is no disagreement in the principle that cytogenetic tests can supply this information. In the present paper I would like to present three questions relating to the significance of cytogenetic abnormalities in which there is not uniform agreement.

The first of these questions is whether or not chromosome abnormalities in the form of chromosome breaks or clastogenic events can serve as an indicator or test system for gene mutations. The basis of this possibility is the high correlation between the ability of an agent to produce gene mutations, and its ability to produce chromosome breakage. These correlations are seen with both x-irradiation and chemicals. In the case of irradiation, it is exemplified by the work of Sparrow (*1*), in which a linear dose response curve is found with both single-hit chromosome abnormalities and gene mutations. With chemicals there is virtually 100% correlation between the ability of a chemical to produce gene mutations and its ability to produce clastogenic events. Some data were summarized a few years ago by Kihlman (*2*).

It should be pointed out that while this general correlation between an agent's ability to produce mutagenic and clastogenic events is true, there are defects in the quantitation and sensitivity of the correlations. Thus, polyfunctional alkylating agents are more effective chromosome breakers than monofunctional alkylating agent, but this is not seen in their effectiveness as mutagens. Also, methylating agents tend to be more effective than ethylating agents as clastogens, but are less effective as producers of gene mutations. There is also no indication that the mechanism of production of clastogenic events and point mutational events is the same. On the contrary, Freese (*3*) has presented evidence that the mechanisms are probably quite different. Furthermore, there are very elegant tests available for gene and point mutations as those described by Ames (*4*). The prime reason for considering clastogenic effects as a possible test for gene mutations, is the aspect of human relevance. Chromosomal studies are the only

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tests currently directly applicable to human *in vivo* testing. The question then, Is this correlation between clastogenic and mutagenic effects sufficient to use as a test for gene mutations because of the high relevance to man? In my view it is. This would of course in no way replace the other tests for gene mutations that have been suggested for screening programs. Rather, after these screening programs have been carried out, at the time of human drug trial or human exposure to a new environmental chemical, human leukocyte chromosomes would be studied in the exposed population. The induction of clastogenic events by any new compound would serve to alert to the possibility that in the human species this agent might produce gene mutations, and this information would be utilized for appropriate cautions, additional testing and evaluation of the risk versus benefit.

The second question to be presented concerns the significance of *in vivo* versus *in vitro* cytogenetic testing. The method of choice for cytogenetic testing is an *in vivo* system, because of the problems of metabolic activation of chemical compounds. However, if biologicals as virus vaccines, viral pesticides, or disease-producing viruses are to be evaluated in screening procedures, special problems are encountered that are currently best solved by an *in vitro* system. This relates to the species specificity exhibited by many viral agents which requires that human cells be used in testing, and an *in vitro* human cell system is the most practical current method. This question then, relates to whether or not the risk of genetic damage from biological material is great enough for these to be included in screening procedures.

The third question to be raised also relates to *in vivo* versus *in vitro* testing. In this case, the question is in relation to the specific type of defect termed a "pseudochiasmata" or a "side-arm bridge." It was at one time felt that this type of abnormality might only represent stickiness and not be a true defect. However, electron microscopy studies (*5*) have demonstrated quite conclusively that pseudochiasmata do represent true abnormalities. It is difficult to recognize pseudochiasmata in metaphase cells of human origin or in mammalian cells that have many chromosomes. These defects are easily recognized, however, in anaphase. As reported elsewhere, in these proceedings (*6*), the anaphase test has not worked well in *in vivo* systems. This test does work very well in *in vitro* systems. This question then, is whether or not the pseudochiasmata type of defect is of sufficient significance to warrant the inclusion of an *in vitro* anaphase system. At the present time this is a very difficult question to decide, since there is very little information available on the genetic significance of pseudochiasmata or side-arm bridges. This question is raised now for future consideration, rather than in the hope of a solution at this time.

Summary

Three questions are presented concerning the significance of cytogenetic abnormalities and test systems for their detection. These are: (1) Should clastogenic activity in an *in vivo* human system be considered a test for gene mutation because of the high correlation between these two events and relevance to man? (2) Should virus vaccines and biologicals be tested *in vitro* with human cells? (3) Are pseudochiasmata or side-arm bridges of sufficient significance in patients to consider *in vitro* anaphase methods for their testing?

In my view, the answers to the first two questions are affirmative, while data for a conclusive answer to question 3 ar enot sufficient at the present time. This decision should be deferred until a later time.

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Discussion

Dr. G. Wolff (*NCTR*): Even though it looked bad for the anaphase system, I must say I was impressed by the presentation on macronucleus system as it showed up when erythrocytes were used. If this sort of system would work we might have a good *in vivo* system there.

Dr. Nichols: I agree completely. The micronucleus test systems on polychromatic red blood cells appears to be an excellent *in vivo* mammalian test system that I believe should be used. I'm saying that human leukocyctes should be used at the time of human exposure to a new compound in addition to these other tests.

Dr. M. S. Legator (*Brown Univ.*): I think we very frequently make the mistake of talking about *in vitro* or *in vivo* and saying we have to use one or the other. Actually this is not what we should be doing. Certainly nobody will ever say that because we do an *in vivo* test we can't do an *in vitro* test. The real issue is, can we handle a screening operation an *in vivo* procedure as well as an *in vitro* procedure and then see what the information generates. Most of the cytogenetic procedures that one will employ will usually and very conveniently handle both; I think that the real problem is that we automatically say to ourselves that the *in vitro* procedures are cheap and fast and therefore we should use a kind of screening pyramid here. In fact, I think this is the wrong way to do it. We should try to do the *in vivo* procedures when they are not completely impractical but also carry out *in vitro* techniques and then assess the overall data and not look at these things as separate categories.

Dr. Nichols: I certainly agree. What I'm really asking, though, is are they important enough to do. I'm not saying that facetiously; I wonder if biologicals are important enough to require evaluation. I think that they probably are. I think that there will probably be better methods in the future for these evaluations, but at the present time an *in vitro* method with human cells is the best answer for testing biologicals as viruses for a potential mutagenic capacity.

Dr. W. G. Flamm (*FDA*): Four of us have worked together on a document that relates to what Dr. Nichols has said. I want to say that I agree completely with him about the utility of the somatic cell cytogenetic methods, insofar as it provides a way of making comparisons from the laboratory animal to man. I think this is a very important thing, one of the few things we have that we can use. With all of our talk about extrapolation I think it is absolutely clear that we want to be able to use it. Now the question is, do we want to apply it across the board; would it be useful, for instance, in food additives, and so on. My own opinion is I have not yet identified those areas where it would be useful, but clearly it is useful for drugs; I think that there is probably unanimity of opinion on that point.

Dr. J. F. Crow (*Univ. of Wisconsin*): What's different in principle between drugs and food additives?

Dr. Flamm: Well, with drugs you can do epidemiology; you can identify exposed populations; you can ascertain levels of exposure and kinds of exposure when you are dealing with a chemotherapeutic situation, if you're in phase 1 or phase 2 of the drug study. With food additives, on the other hand, we're asking the question about a substance which may or may not be harmful, but is ubiquitous, reaches us in relatively trace amounts, either in indirect or direct food additives, or what have you, something on which we cannot do epidemiology because we do not have a defined human population with a defined exposure. The relationship between the cytogenetics and the human, I think, affords us nothing.

Dr. S. S. Epstein (*Case Western Reserve*): I'm not clear what epidemiology has to do with the problem we're talking about. Clearly there are tremendously difficult problems in epidemiological analyses of adverse effects for agents to which massive populations are exposed and to which there are no sharp differential exposures. This is very, very clear. It is much simpler to pick up effects in very small populations which are exposed to single agents than to massive populations which are exposed to a wide variety of small level agents. I don't understand the basic distinction you're trying to make between drugs and other kinds of chemicals, however. First of all, the pharmacologists and toxicologists often use the word drug to imply any kind of chemical agent; in fact, we talk about animal drugs, which are really feed additives, and a wide range of materials ranging from antibiotics to growth hormones or stimulants are treated as animal drugs and residues of them come through into the diet. If you are interested in making comparisons between effects in animals (which I think is a terribly important thing to do) I couldn't agree with you any more that cytogenetics is one of those few areas which allows one to go directly from the animal situation to the human situation. Clearly, one can do the same with food additives and with pesticides as one can for drugs. As far as materials that are already on the market and are in our daily diet, we cannot use cytogenetics in a random population to see what their effects are, but one can use specific human volunteer groups, as is usual in the

development of any food additive or any pesticide and see what happens from the point of view of limited exposures at the same time as one does ones metabolic studies one can do some limited cytogenetic studies. I think it is very important to regard all chemicals as a homogeneous kind of entity and not to create artificial distinctions which reflect, it's true, the realities of difficulties in epidemiological analysis.

Dr. Flamm: I don't want to mislead anybody in this area with my comments where it's possible to do work in this area. I think it should be done, and you've identified some of the areas where it's possible. In fact, specific suggestions would be helpful to the agency that could subsequently be used by epidemiologists, and, as you probably know, attempts are being made to build a program in that area. I would think that the cytogenetic effect is likely to be a part of that. It certainly is going to be considered. But when we come down to GRAS compounds it will be really hard to come up with those matched controls that we would need to ascertain doubling exposures.

Dr. Legator: The thing that I can't quite comprehend is why we need this kind of epidemiological information in man before we declare something a hazard.

Dr. Flamm: I'm saying the exact same thing Dr. Nichols is saying. We're not talking about the requirement that we look at man to say whether the thing constitutes a hazard. I think what Dr. Nichols is talking about is that this is the one way that we have of understanding what relationship exists between what we do in the laboratory and what's happening out in the human population. Now we're not saying whether this is tantamount to making a determination as to the safety or the hazardous nature of the substance.

Dr. S. Abrahamson (*Univ. of Wisconsin*): This looks like an area of agreement. I think that Sam and you are now in agreement and that Marvin and you are now in agreement.

Dr. Legator: The point I thought we were discussing here is, does cytogenetic methodology give indication of a mutagenic event? Now, in that context the only thing I was trying to add is that if we find, as I do believe, that chromosomal abnormalities constitute evidence of genetic hazard, and if we find this with GRAS compounds, i.e., drugs, pesticides, and cosmetics, this is an indication of a hazardous substance and we should take the appropriate action. Perhaps we're all in agreement there.

Dr. V. A. Ray (*Pfizer*): There are two points. First, what can we do when we find in experimental animals or even in man that we have a lot of clastogenic effects but can't match them up with any definite mutation? What do we do when we can't find anything, demonstrate a point mutation or demonstrate by abnormalities in any other system that there is realy a genetic error? That's a concern I think. The second is what happens when you generate all data on somatic cells—a majority of cytogenetic evaluations are on *in vivo* bone marrow, really very little is being done on germ cells? Should we extrapolate those data to the germ cells?

Dr. Wolff: In the first place, we heard on the first day, in response to a question of Dr. Crow in answer to Dr. Cummings, that many of Dr. Russell's specific locus mutations were indeed deletions, which means that these are just subvisible clastogenic effects. They are the equivalent of chromosome breaks but smaller and unlikely to be lethal (to the cell). Thus we can relate now chromosome breakage to an event that will be mutagenic, cause a heritable change. The ratio of these two events, the big ones that we can see (which will kill a cell) to the small ones with which the cell will be viable, is still unknown, but we can get that ratio. Once we have that ratio, it seems to me you can tell in the mouse, for instance, the difference between the things you can see and those that are mutagenic. Dr. Brewen spoke about ways in which you could now make a further transition: to go from the things you can see in the mouse to things you can see in the man. From there, though it may be a bit tenuous, it seems reasonable to assume that the ratio in mouse between the big changes and the small ones might very well hold. There is another thing you asked (which has come up time and time again at this meeting): that's the question regarding work with somatic cells. One of the types of somatic cells that is being studied is spermatogonia. In terms of genetic effects, these are the cells that are at risk for a long time, because these are the ones that keep producing the gametes throughout the generation—30 years if you want to take this as the genetic generation. These are the cells that are going to be exposed over their lifetime, whereas the mature germ cells, sperm, are only going to last about 2 weeks and then be gone; in general, you don't have to have as much concern about those cells as you do about the spermatogonia which will give rise to the mature sperm.

Dr. M. Shaw (*Anderson Hospital*): It doesn't really matter to me whether a person becomes mentally retarded or has a general defect or gets cancer because of a clastogen rather than a mutagen. I think that it's still a hazard.

Dr. E. Freese (*NINDS–NIH*): I'd like to emphasize that the ratio between the point mutagenic effect and the chromosome-breaking effect may be as much as a factor of 10,000 for some agents. For some agents we have low ratios, for others we have high ratios. Although Dr. Nichols' method may be useful, I think we have to make very sure that point mutagenic tests are also done. In addition, Dr. Nichols himself had told me that the measles virus is amazing, because it produces chromosome breaks but does not seem to cause cancer or mutations. I thought the reason for this finding was that any cell which is infected by the measles virus suffers so

many chromosome breaks that it cannot survive. In that case, Dr. Nichols' test might predict more mutagenic harm than actually exists.

Dr. Nichols: I didn't really mean to say that; that is bad communication on my part. I think that a disease like measles is so common in the population—something like 99%—that we just can't tell what its relation is to cancer or mutation. Certainly most of the lymphocytes we see with damage would result in dead cells but not in all of them.

Dr. Mauer (*Hoffman-LaRoche Inc.*): I would like to put a caveat in here. That's the fact that many of the *in vitro* tests are run at rather toxic dosages. Obviously, if you don't take cell survival or some other indication like mitotic index, everything will be clastogenic but not necessarily mutagenic. We can't (operationally at least) measure these small chromosomal effects, and that applies to the *in vitro* situation. Of course, we see a lot in the literature on *in vivo* level at enormous dosages which are toxic in the animal to the bone marrow cells, and that should be taken into consideration.

Dr. B. Ames (*Univ. of California, Berkeley*): It might be interesting to try some of these reactive intercalator-type frame shift mutations in some of these other tests; in other words, to use some activated afltoxins which are easy to make or activated benzpyrene in some of these chromosome break tests.

Dr. Legator: They have been used.

Dr. L. Friedman (*FDA*): On the question of epidemiology, I would think everyone here is in agreement. We do need to have epidemiology for the purpose of surveillance to see whether something's gone wrong or whether we can identify the agent. In the general sense I think that's very important. That's one kind of program for which we need methods and for which we hope the methods will be developed. The use of humans, however, is not epidemiology; this is human study with defined populations and under known areas of exposure. This also should be done whenever we think we have tests that are relevant. It will be inevitable that we make choices, because we can't do everything at the same time. I just want to point out that an essential difference between therapeutic agents and other environmental low level agents is that therapeutic agents or diagnostic agents are used by their very nature at biologically effective levels—else why use them. Because of this difference, and everything else we are concerned about, it would seem to me they would have some priority for testing.

Dr. Nichols: To respond to Dr. Mauer, with respect to clastogenic effects, when human exposure is started with a new agent whether industrial, drug, or food additive, I believe if it's a new agent—even if it's passed all previous tests—if its a clastogen in humans then I would consider it a mutagen. I'm taking the situation where it's passed all the animal testing. We have several good plans which should be used and better tests for gene mutations than clastogenic effects, but I do agree with what Dr. Shaw said about clastogenic effects.